Chapter 11

USE OF *ARTEMIA* AS A FOOD SOURCE FOR AQUACULTURE

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I. INTRODUCTION

Although *Artemia* has been known to man for centuries, its use as a food for the culture of larval organisms apparently began only in the 1930s, when several investigators found that it made an excellent food for newly hatched fish larvae.[1-3] As aquaculture developed in the 1960s and '70s, the use of *Artemia* also became more widespread, due both to its convenience and to its nutritional value for larval organisms. The fact that *Artemia* dormant cysts can be stored for long periods in cans, then used as an off-the-shelf food requiring only 24 h of incubation makes them the most convenient, least labor-intensive, live food available for aquaculture. The nutritional value of *Artemia*, especially for marine organisms, is not constant, but varies both geographically and temporally.[4] During the last decade, however, both the causes of *Artemia* nutritional variability and methods to improve poor-quality *Artemia* have been identified.

This article covers both historical and present usage of *Artemia* in aquaculture, with particular emphasis on the nutritional aspects of this important food. The reader may also wish to consult other recent review articles[4-9] for more in-depth treatment of some of the subjects covered here.

II. *ARTEMIA* SUPPLY AND DEMAND

The history of *Artemia* cyst harvesting and commercialization reveals an interesting evolution. In the 1950s commercial supplies originated from two sources in the U.S., i.e., the salt pans in the Bay of San Francisco, California and the Great Salt Lake, Utah. *Artemia* cysts were marketed at a very low price (less than 10 U.S. dollars per kg) for the aquarium pet trade.[10] With the start of hatchery research in marine fish and crustacean farming in the early sixties, new marketing opportunities were created for *Artemia* cysts. San Francisco Bay, Great Salt Lake, and Chaplin Lake (Saskatchewan, Canada) *Artemia* were the first commercial strains used in aquaculture research and production.

Increased interests in aquaculture research and development around the globe, decreased harvests from the Great Salt Lake (because of weather conditions; see below) and Chaplin Lake, high import taxes in certain third world countries (e.g., 100% in Brazil in 1975),[11] and possible simulation of cyst shortages by certain commercial companies resulted in severe price rises of *Artemia* cysts. By the mid-seventies, wholesale prices ranged from 50 to 100 U.S. dollars per kg.

The dramatic impact of the aggravating cyst shortage on the expansion of hatchery aquaculture of marine fish and crustaceans was repeatedly underlined at international conferences.[12] Third world countries often could not afford to import the expensive cysts. The suggestion was made to abandon *Artemia* and look for alternative solutions to culture food for the early larval stages of fish and crustaceans.[13]

Fundamental and applied research with brine shrimp *Artemia* was initiated at the State University of Ghent in the early 70s. Sorgeloos claimed in 1976 that the cyst shortage was an artificial and temporary problem.[14] This was verified when commercial cyst production began from a number of new sources, including a number of third-world countries. This occurred with the help of international aid organizations, as well as many private companies. By 1980 a change in the *Artemia* cyst situation became obvious. New commercial products were available from Argentina, Australia, Brazil, Colombia, People's Republic of China, France, and Thailand. Prices dropped considerably and market competition was aggressive. A market survey estimated the annual cyst consumption by aquaculture hatcheries was about 60 metric tons.[15,16]

However, new problems arose. The hatching quality of commercial cyst products was inconsistent[17] and the nutritional value of *Artemia* varied among sources and even among

batches.[16] Climatological conditions eliminated cyst production at a number of sites for several years. For example, flooding of the south arm of the Great Salt Lake, Utah, interrupted cyst production for over five years. On the other hand, dilution of the north arm resulted in optimal salinity levels that increased annual cyst harvests to several hundred tons during the mid-80s. By 1988 the increase in salinity in the south arm again resulted in new cyst production.[18] Finally, cyst production in large saltworks was high for a few years following inoculation (e.g., over 10 ton per year in the CIRNE saltworks at Macau, Rio Grande do Norte, Brazil, in the late 70s),[19] only to subsequently drop to insignificant levels due to lack of proper management, e.g., control of nutrient levels, manipulation of cyst induction, etc.

Currently, cyst production, availability, and price are relatively stable, due to efforts in *Artemia* research and development in the first half of the eighties,[4,20,21] application of improved methods for harvesting and processing of cysts, the introduction of *Artemia* cyst quality certificates, and the enhancement of cyst food value via bioencapsulation with particulate or emulsified enrichment diets. The price of cysts is now quality dependent, ranging from $25 per kg for Great Salt Lake cysts (large nauplii, low levels of the essential fatty acid 20:5(n-3) to $80 per kg for cysts characterized by high yield, synchronous hatching, and small nauplii which are pollution free and contain high levels of 20:5(n-3); i.e., >10 mg/g dry weight nauplii.

Since the mid-80s *Artemia* cyst consumption has increased to several hundred tons annually as a result of the worldwide expansion in commercial larviculture of marine fish, shrimp, and prawn. For several aquaculture candidate species it was only in recent years that a successful transition could be made from pilot into commercial larviculture. In view of the increased demands for fish-fry and shrimp postlarvae (considered to be the bottleneck in present-day aquaculture),[22] and the expected extension of the list of new commercially cultured species (e.g., mahi-mahi, grouper, halibut, etc.) *Artemia* cyst demand is expected to increase for years to come. For example, marine fish-fry production in the Mediterranean is expected to increase fivefold by 1992.[23] Although more use can be made of artificial diets as partial substitutes for *Artemia* nauplii,[24] complete substitution is not likely to occur because of nutritional reasons (e.g., palatability, availability, and digestibility), possible interference with water quality, and, last but not least, price difference.

Although present commercial cyst supplies largely meet demands, a larger diversity of commercial sources is urgently needed to ensure more, and especially predictable, quantities of a commercially available product. Today over 70% of all marketed cysts originate from the Great Salt Lake. An exceptionally wet winter in Utah might wipe out the *Artemia* population in the Great Salt Lake for an extended period. More commercial attention should be focused on the development of alternative/complementary sources, which, for example, are available for natural harvesting in the Soviet Union and the People's Republic of China.

Small-scale cyst production in man-made saltworks, although technically very successful in several countries in Southeast Asia and Latin America,[25] are not expected to contribute significantly to world cyst supplies. However, they provide interesting opportunities for local commercial developments, especially in specific third-world countries (e.g., Vietnam)[26] with restricted import opportunities, and where local availability of *Artemia* cysts is the first requirement in consideration of a viable hatchery industry.

Aside from the harvesting of cysts from large natural salt lake/pond systems it is anticipated that more integrated projects of man-managed *Artemia* production and use will be considered. Thanks to the discovery of the high food value of adult *Artemia* biomass as nursery and maturation diet for fish and crustaceans,[4,27,28] several fish/shrimp farming operations are planning to set up units for *Artemia* biomass production which could eventually be modified for cyst production in the event of an imminent cyst shortage.

TABLE 1
Procedure for Decapsulation of *Artemia* Cysts [6,7,30,32,55]

1. Hydrate cysts by placing them in fresh or salt water, bubbled in a separatory funnel for 2 h at 25°C.
2. Collect cysts on 125 μm mesh sieve, rinse, and transfer to hypochlorite solution.
3. The hypochlorite solution, made up of either liquid bleach NaOCl or bleaching powder $Ca(OCl)_2$ (in advance), should consist of 0.5 g active hypochlorite product (activity normally labeled on the package) per g of cysts, plus an alkaline product to keep pH > 10 (0.15 g technical grade NaOH for liquid bleach; either 0.67 g $NaCO_3$ or 0.4 g CaO for bleaching powder; per g of cysts), plus enough sea water to make up the final solution to 14 ml/g of cysts. (Dissolve bleaching powder first, before addition of alkaline product.)
4. Keeping the solution at 15—20° C by addition of ice to the surrounding water bath, add the hydrated cysts and stir for 5—15 min. Check the temperature regularly, since the reaction is exothermic; never exceed 40°C. When cyst color becomes grey (with powder bleach) or orange (liquid bleach), or when microscopic examination shows the chorions to be completely dissolved, remove the cysts, and rinse them with water on a 120 μm screen until the chlorine smell disappears.
5. Totally deactivate the hypochlorite by dipping the cysts (< 1 min) either in 0.1 N HCl or in 0.1% $Na_2S_2O_3$ solution, then rinse again with water.

III. FORMS OF *ARTEMIA* USED IN AQUACULTURE

A. DECAPSULATED CYSTS

The hard shell (chorion) that encysts the dormant *Artemia* embryo can be removed by chemical means in a procedure called decapsulation. This procedure requires hydration of the cysts, removal of the chorion with a hypochlorite solution, and washing and deactivation of the hypochlorite. The resulting decapsulated cysts can then be used immediately or dehydrated for storage. A detailed procedure for decapsulation can be found in Table 1. Decapsulation results in a naked, viable embryo that is approximately 210 to 270 μm in diameter,[29] depending on the strain of *Artemia* being used. The decapsulated cysts can then be hatched into Instar I nauplii or can be fed directly to aquaculture organisms.

The advantages of decapsulation are several. First, the embryos become disinfected during this procedure, so any bacteria associated with the cyst shells are not introduced into the culture tanks.[30] Second, the cyst shells are not introduced into the culture tanks and therefore cannot be ingested by the cultured organisms. Third, the decapsulated cysts contain a higher energy content when fed to a predator than do Instar I nauplii, because they do not have to expend energy in breaking out of the shell.[31] Fourth, the decapsulated cysts are smaller particles than Instar I nauplii (compare dimensions given above with the range of Instar I naupliar lengths of 420 to 520μm for various strains). Finally, if the decapsulated cysts are incubated for hatching as Instar I nauplii, the hatchability is improved, again because no shell breakout is required.[32]

The major disadvantage of decapsulated cysts is that they are nonmoving, nonbuoyant particles. Thus, some aquaculture organisms, like marine fish larvae, that could benefit most from their small size, bacteria- and shell-free nature, and high energy content might have difficulty ingesting them if a sufficient number of the particles is not kept in suspension by extra aeration or circulation of water in the tanks. Perhaps for this reason, the major usage of decapsulated cysts seems to be in penaeid shrimp hatcheries, where the postlarvae are more able to withstand extra aeration and can better capture particles at or near the bottom of the tanks.

B. NEWLY HATCHED NAUPLII

Nauplii in the Instar I-II stages are probably the form of *Artemia* most widely used in aquaculture. In order to optimize utilization of cysts for hatching into nauplii, it is useful to know as much as possible about the hatching characteristics of each batch. Percent hatch (the total percentage of the cysts that actually hatch), hatching efficiency (the number of

TABLE 2
Procedure for Hatching and Harvesting *Artemia*[55,61]

1. Place 5 g of cysts per liter of sea water (temperature 25-30°C; salinity >5 g/l) in a conical container. If low-salinity water (5-10 g/l) is used, or if large quantities of cysts are being hatched, buffer with up to 2 g technical grade $NaHCO_3$/l. pH must remain >8.
2. Add aeration to bottom of conical container to maintain oxygen levels above 2 mg/l until cysts have hatched.
3. Maintain a light level of 1000 lux, at least during the first 3—4 h of incubation.
4. When cysts have hatched, remove aeration and allow nauplii to settle for 5—10 min at bottom of container.
5. Remove nauplii onto a screen of ≤150 μm mesh and rinse them thoroughly. Similarly remove any more nauplii that settle at 5—10 min intervals.
6. Place in a separator box and use a light source to attract nauplii away from the unhatched cysts and debris; collect the nauplii with a pipet and transfer to clean sea water.

nauplii hatched per g of cysts), hatching rate, including T_0 (the time to first hatch) and T_{90} (the time to 90% hatch), and hatching output (dry weight of nauplii hatched per g of cysts) can be determined by the methods of Bruggeman et al.,[32] Sorgeloos et al.,[33] Vanhaecke and Sorgeloos,[34] and Vanhaecke and Sorgeloos,[17] respectively. For certain strains, especially those from the Canadian sulfate lakes, it might also be useful to know the difference in hatching percentage in 5 ppt sea water vs. 35 ppt and with decapsulated vs. untreated cysts.[17] Once these are known, routine hatching should be accomplished in containers with conical bottoms, with an external light source of at least 1000 lux to trigger the hatching mechanism[35] and aeration from the bottom of the cone. Specific procedures for hatching and harvesting *Artemia* nauplii are given in Table 2.

Knowledge of hatching characteristics is important due to the variability among batches found by Vanhaecke and Sorgeloos.[17] Percent hatch was found to vary from about 20 to 90% of the total cysts. This quality criterion for *Artemia* cysts obviously accounts for much of the price differences among *Artemia* batches. A batch with 80% hatch will clearly be worth more than a batch with 20% hatch. Number of nauplii hatching per gram of cysts can vary from <100,000 to >300,000. Time to first hatch was also found to vary from about 13 h to about 20 h, and time to 90% hatch from about 20 h to about 32 h. This is important because the practical aquaculturist normally wishes to harvest the nauplii and feed them to the cultured organisms as soon after hatching as possible. With each passing hour, the nauplii metabolize the yolk for their own energetic needs, therefore decreasing in energy content,[36] increasing in size and mobility, and making it more difficult for the predator to capture them.

Perhaps more important than hatching characteristics of the nauplii is the nutritional quality of those nauplii for the particular organism being fed. It should be stressed that, although *Artemia* nauplii are often a convenient, adequate food for short-term rearing of fish and invertebrate larvae, they may not be a nutritionally complete food for long-term rearing of those organisms. In particular, marine fish and invertebrate larvae appear to have different fatty-acid requirements than do freshwater organisms.[37] This subject will subsequently be covered in more detail; we simply remark that a batch of *Artemia* proven to be a good food for rearing freshwater organisms will not necessarily be good for marine organisms. Furthermore, there is no correlation between good hatching characteristics (e.g., >80% hatch) and good nutritional value. Nutritional value for marine organisms is another quality criterion that accounts for much of the difference in price among *Artemia* batches. Those batches that have an adequate fatty-acid composition for both freshwater and marine organisms can be worth two to three times as much as batches that are adequate only for freshwater organisms.

A prudent strategy for the aquaculturist is to buy a small quantity (e.g., one can) of a particular batch of cysts and test it for hatching and nutritional quality before procuring a large quantity. In some aquaculture operations, *Artemia* cysts are used at a rate of several

kg per day. Thus, cysts may cost tens of thousands of U.S. dollars per year and the quality of the cysts should be verified before purchase or a quality certificate should be requested at purchase (as is commonly done now in Europe).

Newly hatched *Artemia* nauplii are normally fed immediately upon harvesting to the cultured organisms. They may be introduced into the culture tank all at once, or they may be metered in slowly, so that some food is always available in the water. The latter method has the inherent drawback that the nauplii lose nutritional value and increase in size while they are held. One solution is the cold storage of nauplii until they are introduced into the culture tanks.[38] If the nauplii are kept at about 4°C until they are added to the tank, their metabolism is slowed and they retain their nutritional value and newly hatched size for up to 48 h.

C. METANAUPLII

The term metanauplii is given to *Artemia* of Instar stages II through V, or roughly 2 to 5 d after hatching. Most nauplii will use up their yolk reserve and starve to death within 3 to 4 d after hatch, so successful utilization of metanauplii as food requires that they be offered food prior to being fed to predators. This has generally meant feeding them with algae so that the use of metanauplii in aquaculture has been limited. In recent years, however, the introduction of enrichment techniques (see below) has allowed for the much simpler culture of *Artemia* to the metanaupliar stages and these are now commonly used worldwide.

The main restriction on the use of metanauplii is their size (about 500 to 800 μm). Many fish and crustacean larvae are not able to ingest such large particles until several days to weeks after they have started to feed. For predators that are capable of ingesting metanauplii, however, the added nutritional value possibly obtained in algae-fed or enriched *Artemia* can make utilization of this form of *Artemia* especially worthwhile. Furthermore, the greater energy content obtained per particle ingested may reduce the energetic costs of feeding for the predator, as compared to the costs of feeding on newly hatched nauplii that may be smaller than the predator prefers.

D. JUVENILE AND ADULT *ARTEMIA*

These forms of *Artemia*, obtained through intensive *Artemia* culture in the laboratory or extensive culture in ponds, are primarily important as live food for penaeid shrimp aquaculture and are often referred to as *Artemia* "biomass". Methods for culture of *Artemia* have been published elsewhere.[7,13,39] Juvenile and adult *Artemia* can be harvested easily from an intensive culture system by siphoning or draining the tank. They can be harvested from a pond either by a large net pushed or towed by a boat or by a net placed in the flow of water at a pumping station between ponds.[40] The harvested *Artemia* may then be fed live to the predator,[41] frozen,[42] freeze-dried[40] for later usage, or made into a flake diet.[43,44]

Live *Artemia* biomass is apparently a good food both for the growth of penaeid shrimp juveniles in nursery ponds and for the maturation of adult brood stock in hatcheries.[20,41] The location of a salina or man-made *Artemia* production ponds in close proximity to an aquaculture facility can be beneficial to both *Artemia* producers and aquaculturists. For example, in Thailand small-scale salt producers also harvest live *Artemia* biomass as a by-product and sell it to fish and shrimp farmers, transporting it by truck.[41] In a demonstration project in the Philippines, salt, *Artemia*, aquaculture, and agriculture production were all integrated into a single business operation.[45]

Another popular use of live *Artemia* is as a food for tropical and ornamental pet fish.[14,42] The mode of transport for this application is by airplane, as well as by surface vehicles, since the quantities required are smaller.

E. FROZEN AND FREEZE-DRIED *ARTEMIA*

Artemia biomass can be frozen, freeze-dried, or made into a flake diet for storage and

subsequent usage, with relatively little loss of nutritional composition. The frozen form is sold commercially by several companies, which often advertise in aquaculture trade magazines. Use of freeze-dried and flake diet forms has recently been suggested by Guimaraes and Lira do Rego[40] and Janata et al.,[43] although their effectiveness as food (compared to live *Artemia*) has not yet been completely demonstrated.

Frozen adult *Artemia* are normally used for culture of larval American lobsters (*Homarus americanus*), as well as other crustacean and fish species (see review in Léger et al.[4]) Frozen *Artemia* nauplii have also been used for culture of both crustacean[46,47] and fish[48,49] larvae. Some investigators have observed that frozen or freeze-dried nauplii could lead to reduced survival or growth of fish larvae,[50-53] however, perhaps due to the loss of essential nutrients during the thawing process.[54]

IV. FACTORS INFLUENCING THE EFFECTIVENESS OF *ARTEMIA* AS A FOOD FOR AQUACULTURE

A. CYST COLLECTION AND PROCESSING

The collection and processing of cysts is normally done by the commercial supplier and therefore is beyond the control of the aquaculturist. Variability in these procedures is likely to affect hatchability more than nutritional value. Optimal procedures for collection and processing have been given by Sorgeloos et al.[55] Hatchability of cysts is probably decreased the longer they remain on the shore of a pond, which is subject to alternations in drying and rehydration. The cysts should, if possible, be collected directly from the hypersaline pond water, e.g., through the use of collection barriers oriented perpendicular to the predominant wind direction.[55] The collected cysts should then be separated from debris, sand, etc. Optimal hatching will result when cysts are dried in a fluidized bed dryer and packaged in cans under either vacuum or nitrogen.[34]

B. STORAGE OF CYSTS

Cysts should be dehydrated before storage (moisture content preferably <5%), because a moisture content greater than 10% reduces hatchability.[6] Storage under vacuum or nitrogen is important because the presence of oxygen can cause formation of free radicals that reduce hatchability.[56] Properly packaged containers of *Artemia* cysts can be kept at room temperature or can be frozen;[6] however, if frozen, the cysts should be held for 1 week at room temperature before they are incubated in sea water.[57]

It should also be noted that storage of frozen brine shrimp (nauplii or adults) for extended periods in the presence of oxygen, similarly to any other aquaculture food, can result in the oxidation of the long-chain highly unsaturated fatty acids (possibly causing rancidity)[58] and the depletion of vitamin E due to its antioxidant properties.[59]

C. HATCHING OF CYSTS

Problems of hatching variability have been discussed above. A knowledge of the hatching characteristics of a particular batch of cysts and application of that knowledge to daily hatching procedures can greatly increase the effectiveness of usage of that batch in a hatchery. Some standard rules can be applied to maximize the hatching and naupliar survival. First, the container used for hatching should have a conical bottom into which aeration is introduced. Any container with a cylindrical or square bottom will have "dead spots" in which *Artemia* cysts or nauplii will accumulate and deplete the oxygen supply. Aeration in the bottom of a conical container will keep cysts and nauplii continually in suspension. Second, the aeration level should be sufficient to maintain a dissolved oxygen concentration of at least 2 mg/l (preferably 5 mg/l). Third, incubation should be carried out at a maximum concentration of 5 g cysts per 1 of sea water for small volumes (e.g., <20 l) decreasing to

a concentration of 1 g cysts per l for large volumes (e.g., >100 l). Fourth, an external light source is needed, especially during the first few hours of incubation,in order to trigger the hatching mechanism. Fifth, technical grade sodium bicarbonate should be added at a maximum rate of 2 g/l in order to maintain the pH>8.0 throughout the incubation period. Finally, if optimal procedures are followed and cysts still do not hatch well, it may be possible to increase hatching percentage by treatment of the cysts with hydrogen peroxide (e.g., 5 min soaking of dry cysts in a 5% hydrogen peroxide solution).[6,60] For a more complete review of *Artemia* cyst dormancy and hatching, the reader is referred to the recent review article by Lavens and Sorgeloos.[6]

D. HARVESTING OF NAUPLII

Four aspects of harvesting nauplii primarily govern the effectiveness of those nauplii as food: (1) The harvest should take place as soon after T_{90} as possible to ensure maximum nutritional value of the nauplii.[31,36] (2) Nauplii should not be allowed to settle for too long in the bottom of the conical container (i.e., 5 to 10 min maximum), lest they die due to insufficient oxygen. (3) Nauplii should be collected on a submerged fine mesh (<150 μm) screen and rinsed well with minimal mechanical harm to the nauplii before being fed to cultured organisms, in order to prevent the introduction of hatching by-products like glycerol into the culture system. (4) Nauplii should be separated from hatching debris and any dead nauplii (e.g., with a separator box)[61] so that only freely swimming, live nauplii are introduced into the culture system. Feeding dead nauplii that become unavailable to the predator can reduce culture output[62] and foul the water.

E. BIOMETRICS

Difference among *Artemia* geographical strains with regard to size of cysts and nauplii has been well-studied by Vanhaecke and Sorgeloos.[29] Size appears to be genetically determined and cysts collected over time from a particular location are relatively similar in diameter. Cysts from San Francisco Bay and northeastern Brazil tend to be among the smallest available, and those from India, China, Italy, and France, among the largest. Finally, there is a high correlation between cyst diameter and naupliar length, as well as for several other biometric characteristics.

Size of nauplii is often not critical for crustacean larvae, which can capture and manipulate food particles with their feeding appendages. For fish larvae, which have no feeding appendages, the ability to engulf a prey particle in one bite is critical. Fish larvae that receive overly large *Artemia* nauplii may starve because they cannot ingest the particles. For at least one species, a high correlation exists between naupliar length of *Artemia* and larval fish mortality during the first five days after hatching.[63] Some fish species must be fed rotifers as a first food because nauplii from all *Artemia* strains are too large. In those cases, the size of nauplii will determine when the fish can be switched from a rotifer to an *Artemia* diet.

F. BIOCHEMICAL COMPOSITION

Many aspects of the biochemical composition of *Artemia* have been studied and the results will only be summarized here. The reader is referred to the review by Léger et al.[4] for a more extensive treatment of this subject.

The dry weight and individual energy content of Instar I *Artemia* nauplii are strongly related to the size of the cysts and nauplii,[31] as one might expect. Dry weights range from about 1.6 to 3.3 μg per nauplius and energy content from about 0.037 to 0.073 J per nauplius.[31]

Literature reports on proximate composition of nauplii vary greatly, ranging from 37 to 71% protein, 12 to 30% lipid, 11 to 23% carbohydrate, and 4 to 21% ash.[4] Values for adults are 50 to 69% protein, 2 to 19% lipid, 9 to 17% carbohydrate, and 9 to 29% ash.[4]

Fatty-acid composition of *Artemia* is probably the most studied biochemical component, due to the early observations of Watanabe et al.[64] demonstrating that the long-chain essential fatty acids in *Artemia* were especially variable. A recent review[4] of a large data base of published and unpublished fatty-acid profiles revealed that six fatty acids, 16:0, 16:1(n-7); 18:1(n-9); 18:2(n-6); 18:3(n-3); and 20:5(n-3), normally make up about 80% of the total fatty acids in an *Artemia* sample. The saturated and monoene fatty acids of that group, 16:0, 16:1(n-7), and 18:1(n-9), typically comprise 40 to 60% of the total fatty acids. However, it is clear from the work of Watanabe and his colleagues, as well as the work of the International Study on *Artemia*, and subsequent work on *Artemia* enrichment,[4] that the (n-3) series of fatty acids, 18:3(n-3) and 20:5(n-3), determine the nutritional effectiveness of the *Artemia* more than any other single biochemical component. Linolenic acid, 18:3(n-3), is an essential fatty acid for freshwater organisms, and eicosapentaenoic acid, 20:5(n-3), is an essential fatty acid for marine organisms.[37] If both fatty acids are lacking (a very rare phenomenon) the *Artemia* will probably be a poor food for both freshwater and marine aquaculture species. Normally *Artemia* samples contain either (a) 18:3(n-3) levels >20% of total fatty acids and 20:5(n-3) levels <5%, or (b) 18:3(n-3) levels <10% and 20:5(n-3) levels between 5 and 13%. Only the latter *Artemia* batches can be successfully fed to marine organisms, because marine organisms appear to require *Artemia* with 20:5(n-3) levels >5%, but both types can be fed to freshwater organisms.

The fatty acid composition of an *Artemia* batch is environmentally, not genetically, determined. Millamena et al.[65] and Lavens et al.[151] have demonstrated that the fatty-acid profiles of *Artemia* adults, as well as the cysts they produce, strongly reflect the fatty-acid profile of the diet that they were fed. *Artemia* cysts produced in large salt lakes with a single predominant algal species (e.g., Great Salt Lake, Utah) therefore have a more constant fatty-acid profile over time than do cysts from ponds in solar salt evaporation systems, in which algal species can change markedly both temporally and spatially. In recent years, cysts from Great Salt Lake (by the hundreds of metric tons) have contained high levels of 18:3(n-3) and low levels of 20:5(n-3). Thus, the majority of the world's cyst production has been adequate only for culture of freshwater organisms. This has strongly influenced world market prices and led to the commercialization of enrichment products (see below) so that food for marine organisms could be made available.

The amino acid composition of *Artemia* nauplii, by contrast, seems to be remarkably similar from strain to strain,[66] suggesting that it is not environmentally determined in the manner that the fatty acids are. Furthermore, the ten essential amino acids required for fish[67] are normally present in sufficient quantities in *Artemia* nauplii.[66]

The presence of several proteolytic enzymes in *Artemia* developing embryos and nauplii[4,68,69] and the measurement of proteolytic activity in *Artemia* nauplii[70] has led to speculation that the exogenous enzymes in the nauplii play a significant role in breakdown of those nauplii in the digestive tract of larval fish.[4,71] This has become an important question in view of the relatively low levels of digestive enzymes in many first-feeding fish larvae and the inferiority of prepared feeds (vs. live) for marine fish larvae.

Vitamin analyses have been carried out only on San Francisco Bay *Artemia* cysts[72] and adults.[73] Whether or not significant differences exist within or among strains is therefore unknown. The published data indicate that levels of niacin, pyridoxine, and riboflavin in *Artemia* are slightly less than recommended for cold-water fishes.[67]

The levels of several minerals in *Artemia* as reported in the literature have been summarized recently.[4] The mineral requirements of marine organisms are poorly known and may be met by the sea water that they ingest. The main concern about *Artemia* mineral composition is whether they meet the requirements of freshwater organisms in culture, about which our knowledge is also poor. A recent study of variability of 18 minerals and trace elements in *Artemia* cysts revealed that levels of selenium and manganese were the most variable and that selenium in some cases may not be present in sufficient quantity.[74]

V. THE NUTRITIONAL ENHANCEMENT OF *ARTEMIA*

A. SOLUTION TO THE PROBLEM OF NUTRITIONAL DEFICIENCIES

In the previous section it was concluded that the main factor affecting the nutritional value of *Artemia* as a food source for marine larval organisms was the content of the essential fatty acid 20:5(n-3). This highly unsaturated long-chain fatty acid, along with 22:6(n-3), have been proven to be essential for marine fish and crustaceans.[37,75-85] The content of 22:6(n-3) in freshly hatched *Artemia* is low, even nil, while the level of 20:5(n-3) is highly variable (see Table 3 and Léger et al.[4]). In view of the essential fatty acid (EFA) deficiency in *Artemia*, research has been conducted to increase the EFA content (review in Léger et al.[4]). Taking advantage of its continuous nonselective feeding behavior, *Artemia* may be fed any particulate diet when its particle size is below 50 μm.[86] Hence, a diet enriched with EFA 20:5(n-3) and 22:6(n-3) will produce nutritionally more adequate *Artemia*. Léger et al.[87] have demonstrated that feeding cod liver oil-coated micronized rice bran (CLO) fortified with 20:5(n-3) and 22:6(n-3), increased the level of both EFAs in San Pablo Bay *Artemia* (SPB); see Table 4. Prefeeding with rice oil-coated rice bran (RO, lacking 20:5(n-3) and 22:6(n-3)) did not markedly increase the level of either EFA in SPB *Artemia*. The nutritional value of EFA-enriched SPB *Artemia* for the marine crustaceans *Mysidopsis bahia* and *Penaeus stylirostris* was significantly better than the newly hatched SPB nauplii (Table 5). Prefeeding with the EFA-lacking diet (RO) did not enhance the nutritional quality of the nauplii. The enhancement of the dietary value of SPB *Artemia* nauplii was optimal with more refined (AA18) or more concentrated (SEC) enrichment diets (Table 4 and 5) which resulted in higher EFA levels in the enriched *Artemia*. A similar enhancement of the nutritional value of *Artemia* nauplii for marine fish larvae (red seabream) was demonstrated by Watanabe et al.[88] using algae and yeasts containing EFA (Table 6). The best culture results for both fish and shrimp were obtained when enriched *Artemia* nauplii contained not only 20:5(n-3), but also EFA 22:6(n-3); e.g., prefed with ω-yeast, CLO, AA18, and SEC (see Table 4 and 5). This probably explains why better culture results are usually obtained with natural zooplankton such as copepods (e.g., *Acartia* spp., *Eurytemora* spp., *Tigriopus* spp.) than with freshly hatched *Artemia* nauplii.[88-90] Marine zooplankton usually contain high levels of 22:6(n-3).[91] From these above experiments it is apparent that the nutritional value of *Artemia* is governed by its content of EFA (especially 20:5(n-3) and 22:6(n-3), generally referred to as (n-3)HUFA — Highly Unsaturated Fatty Acids), and that the nutritional value of *Artemia* can be enhanced by enriching the nauplii through prefeeding with EFA-rich or enriched diets.

B. ENRICHMENT TECHNIQUES

The finding that the nutritional value of *Artemia* can be improved by prefeeding with EFA, especially (n-3)HUFA-rich diets, has led to the development of a number of enrichment techniques and diets. Their application in marine larviculture is of particular interest because of the variable levels of 20:5(n-3) and the very low levels (if any) of 22:6(n-3) found in *Artemia* nauplii. Léger et al.[4] classified them into four groups: the British technique, with unicellular algae; the Japanese technique, with ω-yeast or emulsions plus baker's yeast; the French technique, with compound diets; and the Belgian technique, with microparticulate diets or self-emulsifying concentrates. Beside the enrichment diet used, the respective techniques vary with respect to hatching conditions, enrichment time (time between hatching and addition of enrichment diet), enrichment period, and temperature. The enrichment levels reported so far are summarized in Table VI of Léger et al.[4] and Tables 7 and 8 of this review.

TABLE 3
Variability in Content of 20:5(n-3) within Several Geographical Strains of *Artemia*

Artemia Geographical Strain	20:5(n-3) Content (range)
San Francisco Bay	0.3—13.3
Great Salt Lake - South Arm	2.7—3.6
Great Salt Lake - North Arm	0.3—0.4
Chaplin Lake (Canada)	5.2—9.5
Macau (Brazil)	3.5—10.6
Bohai Bay (China)	1.3—15.4

Note:: 20:5(n-3) Content given as area percent; i.e., content of 20:5(n-3) as a percentage of total fatty acids.

Data compiled from References 4 and 87.

TABLE 4
Content of (n-3)HUFAs in Enrichment Diets and *Artemia* Preparations

	20:5(n-3)		22:6(n-3)		(n-3)HUFA	
	Area %	mg/g	Area %	mg/g	Area %	mg/g
Enrichment diets						
CLO	8.0	6.3	10.0	5.2	20.9	14.2
RO	—	—	—	—	Tr	Tr
Artemia preparations						
SFB—newly hatched	9.3	11.8	0.2	0.3	11.5	14.6
SPB—newly hatched	0.5	0.5	—	—	0.7	0.8
SPB—starved 24 h	1.4	1.1	0.6	0.4	3.5	3.0
SPB—CLO-enriched 24 h	6.3	7.3	1.5	1.9	8.9	10.1
SPB—RO-enriched 24 h	0.9	0.8	—	—	1.9	1.9

Note: Data expressed both as area percent (i.e., (n-3)HUFA as a percentage of total fatty acids) and as mg (n-3)HUFA/g dry weight of tissue. Tr, trace; —, not detected Σ (n-3)HUFA > 20:3(n-3); CLO, cod liver oil; RO, rice oil; SFB, San Francisco Bay; SPB, San Pablo Bay.

Data from Reference 87.

TABLE 5
(n-3)HUFA Content of San Francisco Bay and San Pablo Bay *Artemia* Nauplii—Results of Culture Tests for *Mysidopsis bahia* and *Penaeus stylirostris* Fed on Those Diets

	SFB *Artemia*	SPB *Artemia*				
	Newly hatched	Newly hatched	RO-enriched	CLO-enriched	AA18-enriched	SEC-enriched
(n-3)HUFA content (area%)						
20:5(n-3)	9.3	0.2	0.9	6.3	8.2	9.9
22:6(n-3)	0.2	—	—	1.5	1.5	5.9
Σ (n-3)HUFA	11.4	0.7	1.9	8.9	10.6	17.8
Culture results (*M. bahia*)						
Survival (%)	93.3	62.0	60.0	75.0	92.5	95.8
Ind. length (μm)	5532	4587	4285	5029	5375	5254
Ind. dry weight (μg)	354	198	188	259	259	323
Culture results (*P. stylirostris*)						
Survival (%)	47.5	34.0	—	—	45.7	63.9
Ind. wet weight (mg)	1.8	1.7	—	—	2.0	2.7

Note: AA18 and SEC are commercial enrichment products; RO, rice oil; CLO, cod liver oil; Σ (n-3)HUFA >20:3(n-3).

Data from References 85, 87, and 106.

1. The British Technique

In this technique developed by Forster and Wickins[92] and Wickins,[93] algae (*Isochrysis galbana*) are used for enriching *Artemia* nauplii. A density of 10,000 nauplii per liter of sea water containing 300 cells/ml is applied during 24 h for improving the nutritional value of *Artemia* for prawn larvae (*Palaemon serratus*). No data are available on the enrichment levels obtained applying this technique. Using *I. galbana* (T-Iso), Sivertsen[94] obtained a maximum enrichment level of 5.5 mg/g (dry weight basis) after 12 h; this level dropped to 2.6 mg/g after 125 h. The disadvantages of using algae are that one has to maintain continuous cultures and that the (n-3)HUFA content in algae is variable.[95] Recently, Walford and Lam[96] proposed the use of microcapsules (AR 121, Frippak, United Kingdom) containing a high percentage of total lipids as a substitute for algae in enriching *Artemia* nauplii. Total (n-3)HUFA buildup in *Artemia* nauplii reached a maximum of 16.9% (percent of total fatty acids) after a 48-h enrichment. Rimmer and Reed[97] reported enrichment levels up to 12%, (20:5(n-3), 22:5(n-3), and 22:6(n-3) of total fatty acids) using Frippak microcapsules. They obtained better culture results with *Lates calcarifer* when using *Artemia* enriched in this way. Deru et al.,[98] using the same product during a 4-h enrichment, obtained a maximum (n-3)HUFA enrichment of 6.8% in *Artemia*. The dietary value for *Macrobrachium rosenbergi* larvae of these enriched *Artemia* was not significantly different from that of *Artemia* containing 6.0% (n-3)HUFA without enrichment.

2. The Japanese Technique

The "indirect method" as developed by Watanabe et al.[64,90,99] at first applied algae (*Chlorella minutissima*) for enrichment, similar to the British technique. Enrichment levels in *Artemia* obtained with this algae reached 15.5% (n-3)HUFA of total fatty acids. A similar procedure was adopted using the so-called ω-yeast as an alternative to algae. The (n-3)HUFA buildup after 24 h reached 13.8% of total fatty acids. This ω-yeast, an (n-3)HUFA-fortified baker's yeast, was developed by Imada et al.[100] and offers the advantage of having a less variable EFA content than does algae. The disadvantage of using this product is that it has to be used in a fresh, living condition. The "direct method" developed by Watanabe et al.[99,101] requires emulsified fish oils (e.g., cuttle-fish liver oil and pollock oil) and an (n-3)HUFA methylester mixture. The emulsion is fed to the *Artemia* nauplii which ingest and accumulate the droplets. Reported (n-3)HUFA levels attained with this method is 1.01% or 10.1 mg/g *Artemia* (wet or dry weight basis not stated). Watanabe and colleagues concluded that *Artemia* containing at least 0.3% (n-3)HUFA (dry or wet basis not stated) may be a satisfactory single feed for marine fish, *Pagrus major, Paralichthys* sp., and *Oplegnathus* sp.

3. The French Technique

Robin et al.[102] developed an enrichment technique applying a composed diet (*Spirulina* powder, yeast, amino acids, vitamins, cholesterol, and fish oil) during a 48-h prefeeding period. Later, Robin et al.[103] and Robin[104] improved this technique by splitting up the enrichment period in a 48-h prefeeding, followed by a short-term (30 min) enrichment bath, using a diet based on fish autolysate, fish oil, vitamins, and minerals. (n-3)HUFA buildup in *Artemia* reached 16 mg/g (dry weight basis). Higher levels up to 25 mg/g (dry weight basis) were reported by Robin et al.[105] through the use of higher dietary oil concentrations in the prefeeding diet. Better culture results were obtained when *Artemia*, enriched in this manner, were fed to seabass (*Dicentrarchus labrax*) and especially to turbot (*Scophthalmus maximus*). Recently, Sivertsen et al.[94] reported enrichment data obtained with a composed diet containing fish meals and oils. A maximum level of 10.6 mg/g (dry weight basis) was obtained in *Artemia* after a 12-h enrichment. When using micromilled pure squid meal, a maximum level of (n-3)HUFA was attained after 96 h (15.4 mg/g), while the level after a

TABLE 6
Content of (n-3) HUFAs in *Artemia* from Canada and San Francisco Bay (SFB) — Effect of Enriched SFB on Survival and Growth of Red Sea Bream Juveniles

	Canadian *Artemia*	San Francisco Bay *Artemia*		
	Newly hatched	Newly hatched	Fed *Chlorella* for 24 h	Fed ω-yeast for 24h
(n-3)HUFA content				
20:5(n-3)	5.2	1.6	3.2	3.4
22:6(n-3)	—	—	—	
Σ (n-3)HUFA	5.8	2.4	4.1	5.1
Fish culture test				
Survival (%)	68.4	43.4	66.8	86.4
Survival after activity test (%)	37.5	24.1	46.1	50.0
Final length (mm)	9.57	10.13	11.13	11.67

Note: Data from Reference 88.

TABLE 7
Summary of Enrichment Procedures for *Artemia* Nauplii, (n-3)HUFA Content in *Artemia*, and Results of Comparative Culture Tests with Enriched *Artemia*[a]

Artemia source	Hatching		Pre-enrichment			Enrichment			(n-3)HUFA content						Culture test		Ref.
									20:5(n-3)		22:6(n-3)		Σ (n-3)HUFA				
	t	T	Diet	t	T	Diet	t	T	%	mg/g	%	mg/g	%	mg/g	Species	Performance	
SPB (1628)			na			AA18, in hatching medium	46	30	—	2.2	—	3.1	—	9.6	*Dicentrarchus labrax*	+	147
GSL-NA			na			AA18, in hatching medium	46	30	—	3.4	—	2.3	—	6.7	*D. labrax*	+	147
GSL-NA	22	25	na			na			—	0.3	—	—	—	0.5	*D. labrax*	−	147
SPB (1628)	22	25	na			na			—	0.5	—	—	—	2.6	*D. labrax*	−	147
RAC	26	25	na			na			—	10.6	—	—	—	11.2	*D. labrax*	+	147
RAC	T_{90}	28	na			na			7.2	—	0.1	—	—	—	*Spurus aurata*	+	111
Bohai Bay (China)	T_{90}	28	na			na			—	—	—	—	—	—	*S. auruta*	+	111
GSL	T_{90}	28	na			na			0.3	—	—	—	—	—	*S. auruta*	−	111
SPB	T_{90}	28	na			na			0.5	—	—	—	—	—	*Sparus aurata*	−	111
GSL			na			AA18, in hatching medium	48	28	2.1	—	1.4	—	—	—	*S. auruta*	o	111
SPB						AA18, in hatching medium	48	28	1.8	—	2.6	—	—	—	*S. auruta*	o	111
RAC	T_{90}	28	na			na			7.2	—	0.1	—	—	—	*Penaeus serratus*	+	111
GSL	T_{90}	28	na			na			0.3	—	—	—	—	—	*P. serratus*	o	111
SPB	T_{90}	28	na			na			0.5	—	—	—	—	—	*P. serratus*	o	111
GSL			na			AA18, in hatching medium	48	28	2.1	—	1.4	—	—	—	*P. serratus*	±	111
SPB			na			AA18, in hatching medium	48	28	1.8	—	2.5	—	—	—	*P. serratus*	o	111
RAC	T_{90}	28	na			na			7.2	—	0.1	—	—	—	*P. adspersus*	+	111
GSL	T_{90}	28	na			na			0.3	—	—	—	—	—	*P. adspersus*	±	111
SPB	T_{90}	28	na			na			0.5	—	—	—	—	—	*P. adspersus*	±	111

TABLE 7 (continued)
Summary of Enrichment Procedures for *Artemia* Nauplii, (n-3)HUFA Content in *Artemia*, and Results of Comparative Culture Tests with Enriched *Artemia*[a]

Artemia source	Hatching		Pre-enrichment			Enrichment			(n-3)HUFA content						Culture test		Ref.
									20:5(n-3)		22:6(n-3)		Σ (n-3)HUFA				
	t	T	Diet	t	T	Diet	t	T	%	mg/g	%	mg/g	%	mg/g	Species	Performance	
GSL			na			AA18, in hatching medium	48	28	2.1	—	1.4	—	—	—	*P. adspersus*	+	111
SPB			na			AA18, in hatching medium	48	28	1.8	—	2.5	—	—	—	*P. adspersus*	±	111
Brazil (CIRNE)	48	24	na			na			—	—	—	—	6.8	9.2			105
Bohai Bay (China)	48	24	na			na			—	—	—	—	11.7	11.0			105
France	48	24	na			na			—	—	—	—	6.9	7.6			105
France	48	24	G	48	24	na			—	—	—	—	8.5	5.4			105
SFB (2557)	48	24	na			na			—	—	—	—	5.7	8.6			105
SFB (2557)	48	24	G	48	24	na			—	—	—	—	12.7	8.4			105
SFB (2557)	48	24	G	48	24	J			—	—	—	—	14.9	15.9			105
SFB (2557)	48	24	L	48	24	na			—	—	—	—	10.1	10.2			105
SFB (694)	48	24	na			na			—	—	—	—	4.0	5.8			105
SFB (694)	48	24	J	24	24	na			—	—	—	—	7.5	13.0			105
SFB (694)	48	24	G	48	24	na			—	—	—	—	12.2	9.0			105
SFB (694)	48	24	L	48	24	na			—	—	—	—	14.4	14.0			105
SFB (694)	48	24	M	48	24	na			—	—	—	—	16.6	22.3			105
GSL	48	24	na			na			—	—	—	—	4.0	5.2			105
GSL	48	24	G	48	24	na			—	—	—	—	8.9	9.0			105
GSL	48	24	G	48	24	J			—	—	—	—	10.9	12.3			105
GSL			na			na			—	0.5	—	0	—	—	*P. vannamei*	−	148
GSL			na			SELCO (after separation)	24	28	—	21.3	—	12.7	—	—	*P. vannamei*	+	148
AF	24	25	na			na			5.3	7.7	—	—	—	—	*D. labrax*	+	129
GSL	24	25	na			na			0.3	0.4	—	—	—	—	*D. labrax*	−	129

SFB	24	25	na			na			1.9	2.3	—	—	—	—	*D. labrax*	±	129
GSL	24	25	na			SELCO (after separation)	24	25	4.9	8.1	3.1	5.1	—	—	*D. labrax*	+	129
Bohai Bay (China)	48	25	na			na			13.0	13.0	—	—	—	—	*S. aurata*	±	130
Bohai Bay						SELCO (in hatching medium after decapsulation)	48	25	12.3	17.9	3.6	5.2	—	—	*S. aurata*	+	130
Bohai Bay	48	—	na			na			10.8	—	—	—	10.8	—			96
Bohai Bay	48	—	Baker's yeast	20	—	na			11.2	—	0.1	—	11.4	—			96
Bohai Bay	48	—	Baker's yeast	20	—	AR121 (Frippak, 5 million micro capsules/ml)	8	29	12.2	—	3.6	—	16.9	—			96
							24	29	12.6	—	1.2	—	14.4	—			96
							48	29	15.5	—	0.7	—	16.9	—			96
Aquarium products			starved	24	—	BOOSTER (Frippak) 0.3 g/l			7-9	—	1-2	—	—	—	*Lates calcarifer*	+	97
Aquarium products			starved	24	—	na									*L. calcarifer*	–	97
EG			na			na			—	3.2	—	—			*Hippoglossus hippoglossus*	–	94,149
EG						Squid meal 0.2 g/l/d	6	28	—	3.6	—	—			*H. hippoglossus*	±	94,149
							12	28	—	4.5	—	2.5					
							24	28	—	4.6	—	1.0					
							48	28	—	5.9	—	1.6					
							72	28	—	8.1	—	2.8					
							96	28	—	10.1	—	4.7					
							125	28	—	9.0	—	1.8					
EG						SUPERSELCO (0.3 g/l, 2x/d)	6	28	—	4.0	—	---			*H. hippoglossus*	o	94,149
							12	28	—	8.7	—	6.9					
							24	28	—	15.1	—	16.1					
							48	28	—	20.3	—	21.3					
							72	28	—	22.7	—	21.1					

TABLE 7 (continued)
Summary of Enrichment Procedures for *Artemia* Nauplii, (n-3)HUFA Content in *Artemia*, and Results of Comparative Culture Tests with Enriched *Artemia*[a]

Artemia source	Hatching		Pre-enrichment			Enrichment			(n-3)HUFA content						Culture test		Ref.
									20:5(n-3)		22:6(n-3)		Σ (n-3)HUFA				
	t	T	Diet	t	T	Diet	t	T	%	mg/g	%	mg/g	%	mg/g	Species	Performance	
EG						Squid meal plus SUPERSELCO (3:1)	6	28	—	3.2	—	—					94,149
							12	28	—	5.6	—	4.0					
							24	28	—	6.8	—	4.4					
							48	28	—	13.6	—	12.4					
							72	28	—	15.8	—	17.8					
							96	28	—	16.5	—	19.6					
							125	28	—	15.0	—	16.1					
EG						Special composed diet (SINTEF) 0.2 g/l/d	6	28	—	5.0	—	2.0					94,149
							12	28	—	6.5	—	3.4					
							24	28	—	5.9	—	1.7					
							48	28	—	5.9	—	2.3					
							72	28	—	6.8	—	2.2					
							96	28	—	6.6	—	1.6					
							125	28	—	6.3	—	1.1					
EG			na			*Isochrysis galbana*(T-Iso); 0.1 g/l/d	6	28	—	3.9	—	0.2					94,149
							12	28	—	3.8	—	0.9					
							24	28	—	3.6	—	0.5					
							48	28	—	2.6	—	0.5					
							72	28	—	2.3	—	0.6					
							96	28	—	2.2	—	0.5					
							125	28	—	2.0	—	0.4					
GSL	24	28	na			na									*Lates calcarifer*	–	132
GSL	24	28	na			SELCO after separation	24	28							*L. calcarifer*	+	132
GSL	24	28	na			na			3.0	3.4	0.3	0.4	3.4	3.9			107

GSL			na	SUPERSELCO after separation	6	28	7.4	11.7	5.1	8.1	13.4	21.7			
					12	28	10.3	18.4	6.7	12.2	18.0	32.7			
					18	28	13.4	25.0	9.5	18.0	24.5	46.1			
					24	28	16.3	31.7	11.4	22.1	30.2	56.0			
GSL	24	28	na	na			3.7	4.8	0.5	0.7	4.3	5.6	*Macrobrachium rosenbergii*	–	127
GSL				Emulsified coconut oil	24	28	2.3	4.0	0.4	0.8	2.8	4.9	*M. rosenbergii*	–	127
GSL				SUPERSELCO after separation	24	28	17.3	33.5	11.4	22.0	30.9	66.2	*M. rosenbergii*	+	127

Note: The symbols +, o, ±, −, refer to relative values; i.e., very good, good, average, and poor culture results, interpreted from the data given. Abbreviations: t, time period; T, temperature in °C; %, quantity of fatty acid as a percentage of total fatty acids (area%); mg/g, mg fatty acid methyl ester per g dry weight of tissue; Σ (n-3)HUFA, sum of (n-3) highly unsaturated fatty acids (note: this generally refers to the sum of (n-3) fatty acids with 20 or 22 carbons and 3 or more double bonds); na, no preenrichment or enrichment diet was applied; diets G, J, L, M, refer to Table 8; SPB, San Pablo Bay; SFB, San Francisco Bay; GSL-NA, Great Salt Lake, North Arm; RAC, Reference *Artemia* Cysts; AF and EG Artemia Systems, SA, commercial product. AA18, SELCO, and SUPERSELCO are commercial enrichment diets of Artemia Systems NV/SA, Belgium.

[a] These are additions to Table VI in Léger et al., *Oceanogr. Mar. Biol. Ann. Rev.*, 24, 521, 1986.

TABLE 8
Composition (%) of Preenrichment and Enrichment Diets for Which Abbreviations (G, J, L, M) are Given in Table 7

	Preenrichment diets (48 h)			Enrichment bath (0.5 h)
	G	L	M	J
Brewer's yeast	89.4	83.4	73.4	—
Cod liver oil	4	10	20	10
Fish autolysate	—	—	—	73
Choline chloride	2	1	1	4
DL-Methionine	1	2	2	2
Vitamin premix	—	3.6	3.6	—
Vitamin mineral premix	3.6	—	—	11

24-h enrichment was 5.9 mg/g. Replacing 25% of the squid meal ration with an emulsified enrichment diet (SUPERSELCO, Artemia Systems, Belgium) the values reached 38.0 mg/g and 12.0 mg/g, respectively. These authors also reported total amino acid contents in the enriched *Artemia*: 378.2 mg/g (dry weight) using SUPERSELCO during 24 h; 383.8 mg/g using squid meal; 419.4 mg/g using squid meal + SUPERSELCO; 413.9 mg/g using the compound diet; and 407.3 mg/g using *Isochrysis galbana*. The amino acid composition was not significantly different among enrichment diets.

4. The Belgian Technique

This technique consisted at first of prefeeding EFA-enriched microparticles, e.g., micronized rice bran coated with fish oils;[85] compound analog AA18.[85,106] *Artemia* enriched in this manner contained up to 15.1 mg (n-3)HUFA per gram (dry weight basis). Because of the complexity and cost for preparing these products, a new technology was applied for producing a more effective enrichment diet under the form of a self-emulsifying concentrate.[87] This diet is a self-dispersing complex mixture of mainly (n-3)HUFA sources, vitamins, carotenoids, and phospholipids. Upon dilution in sea water, finely dispersed stable microglobules are formed which are readily ingested by *Artemia* and which bring about EFA-enrichment levels which largely surpass the values reported in literature (e.g., 38.3 mg/g to 53.5 mg/g after 24 h and up to 87.6 mg/g after a 48-h enrichment; see Table VI of Léger et al.[4] and Tables 7 and 8 of this review). Different application regimes are proposed for applying the self-emulsifying concentrates to the hatching medium or after separation of the hatched nauplii.[85]

The high enrichment levels obtained with this technique are the result not only of an optimal diet composition and presentation but also of proper enrichment procedures; e.g., nauplii must be transferred or exposed to the enrichment medium as soon as possible before first feeding. In this way, the nauplii begin feeding immediately after the opening of the alimentary tract (Instar II stage). Furthermore, the size increase occurring after hatching during enrichment will be minimal. *Artemia* enriched according to previous procedures reach >900 μm, whereas here, higher enrichment levels are obtained in nauplii measuring 660 μm (12-h enrichment) to 790 μm (48-h enrichment).

Recently a dry form of the self-dispersing concentrate has been proposed with levels obtained in *Artemia* of between 30 mg/g and 60 mg/g, dry weight.

C. (n-3)HUFA REQUIREMENTS

Several studies have shown the beneficial effect of feeding (n-3)HUFA-enriched nauplii to several species of marine fish and crustaceans.[4,107] However, no study has clearly indicated the requirements for (n-3)HUFA for one species or another. Léger et al.[4] summarized I.S.A.

(International Study on *Artemia*) feeding tests with several strains of *Artemia* for several species. They concluded that high survival and growth of marine species are obtained with *Artemia* nauplii containing at least 4% 20:5(n-3) of total fatty acid methyl esters (or about 4 mg/g on a dry weight basis), while levels below 3% consistently yielded poor results. From their experiments Watanabe and colleagues concluded that *Artemia* containing at least 0.3% (n-3)HUFA (dry or wet basis not stated) may be a satisfactory single feed for marine fish. They added, however, that *Artemia* enrichment should always be applied since lipid contents in *Artemia* gradually decrease after hatching.[99]

Recently, it was found that (n-3)HUFA requirements may differ considerably among marine species. Halibut (*Hippoglossus hippoglossus*) and dolphin fish (*Coryphaena hippurus*) larvae appeared to require more than other species such as sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*).[107] Significant increases in survival and growth were obtained only when fish were fed highly-enriched *Artemia* nauplii containing about 50 mg/g. Based on this knowledge, the Working Group on the Mass Rearing of Juvenile Fish (Mariculture Committee of the International Council for the Exploration of the Seas) in 1988, recommended the initiation of a series of studies in order to better identify the qualitative and quantitative requirements for (n-3)HUFA in marine larval fish. Three tasks are currently being conducted: (1) cataloging all existing information; (2) identifying (n-3)HUFA requirements through feeding tests using three enrichment diets containing different levels of (n-3)HUFA; (3) intercalibrating (n-3)HUFA determination methods by gas chromatography.

D. OTHER APPLICATIONS OF ENRICHMENT

Besides EFAs (especially (n-3)HUFA), other nutrients can be incorporated in *Artemia*. Watanabe et al.[8] reported on the accumulation of fat soluble vitamins (especially vitamin A and vitamin E) in *Artemia* during a short-term (9 h) enrichment period. Vitamin A levels increase from below 1 IU/g (wet weight basis) to over 16 IU/g within 9 h enrichment and vitamin E levels increase from below 20 mg/g (wet weight basis) to about 250 mg/g in freshly hatched and up to over 600 mg/g in starved metanauplii, 52 h old.

Besides nutrients, other components may be incorporated as well. Nin et al. (cited by Léger[107]) report on the use of *Artemia* nauplii for administering therapeutics to fish larvae. They demonstrated the use of *Artemia* nauplii enriched with chloramphenicol for *Cichlosoma nigrofasciatum*. Caudal tissue levels of chloramphenicol reached 3 to 4 ppm after 1 h feeding of 24-h enriched nauplii (Figure 1). Curative and preventive treatment of larvae through the food chain may be much more effective than treating the relatively large volume of culture water surrounding them.

The enrichment techniques and diets developed for *Artemia* may also be applied for adult *Artemia* and other live food sources such as rotifers and nematodes.[107-109]

VI. SPECIFIC APPLICATIONS OF *ARTEMIA* FOR FEEDING AQUACULTURE SPECIES

A. PENAEID SHRIMP CULTURE

Artemia is generally used for feeding the late larval and postlarval stages of penaeids. Freshly hatched nauplii are usually offered beginning with the first mysis stage, and sometimes earlier at the zoea-mysis molt. Some authors recommend introduction of *Artemia* even during the second zoea stage.[110] However, penaeids are usually fed algae prior to the *Artemia* and undergo a several-day weaning period when both foods are given. Thus, addition of *Artemia* too early in the life cycle can result in competition for the algae food between the uneaten *Artemia* and the penaeids. A convenient solution may be the early administration of killed nauplii or decapsulated *Artemia* cysts, as suggested by Mock et al.[46] and Wilkenfeld et al.[110]

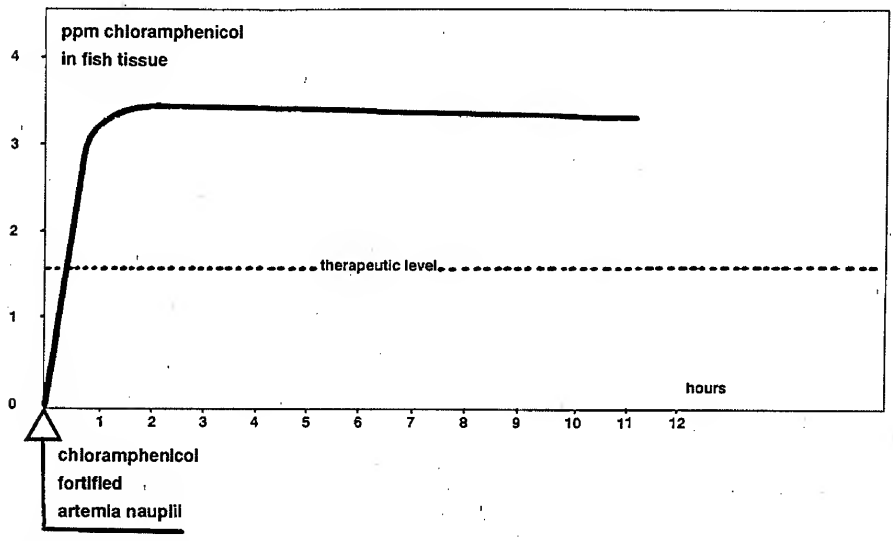

FIGURE 1. Chloramphenicol levels in fish larvae caudal tissue fed one single ration of 24 h-fortified *Artemia* nauplii.

Little information is available on the nutritional value of different strains of *Artemia* for penaeid shrimp. Léger et al.[85] demonstrated that San Francisco Bay (236 2016) *Artemia* nauplii performed significantly better than San Pablo Bay (1628) nauplii. The nutritional value of the latter could be improved by (n-3)HUFA enrichment. In addition, the (n-3)HUFA content of the diet (algae or substitutes) offered during the first developmental stages significantly affected the susceptibility of the larvae to the poor nutritional quality of the San Pablo Bay *Artemia* nauplii. For example, the negative effects of feeding these *Artemia* was aggravated when the animals had previously been fed an (n-3)HUFA-poor diet while no negative effects were noticed when an (n-3)HUFA-rich diet was administered during the earlier stages. Feeding enriched Great Salt Lake *Artemia* nauplii also resulted in better survival and growth in *Penaeus vannamei* postlarvae.[87] When these postlarvae were subsequently fed an artificial diet without transition from a live to a dry diet, better food acceptance, growth, and survival were recorded than in those postlarvae which were previously fed with (n-3)HUFA-poor *Artemia*.

Amat et al.[111] compared the nutritional value of different *Artemia* strains for *Penaeus kerathurus, P. serratus* and *P. adversus*. The best growth and survival of shrimp were obtained with *Artemia* strains containing the highest HUFA levels, although significant differences were not always detected. *P. adversus* larvae grew even better on *Artemia* containing only low levels of (n-3)HUFA (Great Salt Lake and San Pablo Bay *Artemia*). Ongrown *Artemia* have been used successfully for postlarval feeding of *Penaeus monodon*,[112-114] *P. kerathurus*,[115,116] *P. japonicus*,[40,117,118] and *P. aztecus*.[119] Yashiro[114] compared growth and survival in *P. monodon* postlarvae reared on *Artemia* of progressively larger size, fed with different diets (wheat flour, rice bran, and milled rice). Freshly hatched *Artemia* supplemented with cooked mussel meat were fed in the control treatment. Growth after 20 d was significantly better in the treatments fed ongrown *Artemia* (more than double the weight gain), while survival was best in the control treatment, though not significantly better than those shrimp fed *Artemia* grown on milled rice.

Guimaraes and Lira do Rego[40] successfully used ground and sieved freeze-dried and liquefied fresh *Artemia* biomass collected from the saltworks in Macau (Brazil) as a food

source for larval and postlarval *P. japonicus* and *P. aztecus*. *Tetraselmis chuii* and baker's yeast were fed during the Nauplius V and Protozoea I stages only while *Artemia* biomass was offered from Protozoea I stage till harvest (PL VII—PL IX). Following this method, survival averaged 59% over a 3-year period.

B. FRESHWATER PRAWN, *MACROBRACHIUM* SP.

The culture of freshwater prawn larvae depends heavily on the use of *Artemia* nauplii, which is the most successful diet throughout the larval rearing period, and after one week usually in combination with prepared diets.[120-124] In contrast to penaeid shrimp, *Macrobrachium* can initially be fed with freshly hatched *Artemia* nauplii. Sick and Beaty[125] demonstrated that naupliar concentration should be higher than 0.1 nauplii/ml to assure proper ingestion. The same authors found that energy intake in *M. rosenbergii* was directly proportional not only to *Artemia* concentration but also to *Artemia* size. They demonstrated that Stage VIII prawn larvae attained a maximum energy ingestion of 0.0066 cal/mg animal dry weight/h when fed 0.7 mm *Artemia* metanauplii; 0.062 when fed 1.5 mm *Artemia* larvae; and 1.014 when fed 5.5 mm *Artemia* juveniles.

Only a few authors have studied the (n-3)HUFA requirements in *Macrobrachium* sp. D'Abramo and Sheen[126] demonstrated significantly better growth in juvenile prawn with a HUFA-supplemented experimental diet. Deru et al.[98] could not identify extrapolation of this HUFA requirement to the larval stages. They compared unfed *Artemia* nauplii containing 0.3% and 6.0% (n-3)HUFA, and 4-h enriched *Artemia* (Booster, Frippak, United Kingdom) containing up to 6.9% 20:5(n-3) + 22:6(n-3). Using highly enriched *Artemia* nauplii (30.9% (n-3)HUFA, 66.2 mg/g dry weight; SUPERSELCO, Artemia Systems Societé Anonyme, Belgium), Devresse and Rasowo[127] recently demonstrated significant differences in both growth and survival of *M. rosenberghii* larvae using (n-3)HUFA-fortified vs. control *Artemia* nauplii. The first postlarvae appeared on day 16 in the enriched *Artemia*-fed larvae and on day 19 in the control treatment. Metamorphosis was completed on day 22 and day 25, respectively.

C. MARINE FISH CULTURE

The larvae of several species of marine fish, e.g., bream, bass, and flatfish, can only be offered an *Artemia* diet after an initial week on a smaller prey, primarily the rotifer, *Brachionus plicatilis*. In contrast to crustacean larvae, marine fish larvae are cultured on *Artemia* for a much longer period of time, e.g., 20 to 40 d. *Artemia* cyst consumption is also among the highest in marine fish larviculture and ranges from 200 to 500 g per 1000 of the fry produced. *Artemia* biomass in live or frozen form is often applied as a transitional diet for the fry when weaned from a live to an inert food. With the development of better artificial diets, weaning can be successfully applied progressively earlier during the larval development of different fish species.

The variability of the nutritional value of *Artemia* nauplii as a food source for marine larval fish has been well documented.[12] Application of HUFA enrichment of the *Artemia* diet has had a significant effect in marine fish larviculture;[12] i.e., it resulted in increased survival and less variability in fish hatchery production. The latter is especially important since it was the missing link in the development of commercial production. Furthermore, the quality of the fry in terms of stress resistance, better pigmentation, reduced deformities, more successful swimbladder inflation, and increased vigor appears to be directly correlated with (n-3)HUFA enrichment of their larval diet.[128]

The survival of European sea bass (*Dicentrarchus labrax*) appears strongly correlated with the content of 20:5(n-3) in *Artemia* nauplii, while growth is highly correlated with 22:6(n-3) content. All larvae fed unenriched Great Salt Lake *Artemia* died within 35 d, while 25% of those fed (n-3)HUFA-enriched Great Salt Lake *Artemia* survived for 42 d.[129]

For good growth and survival in European sea bream (*Sparus aurata*), the food regime of rotifers and brine shrimp should contain high levels of both 20:5 and 22:6(n-3). Best resistance to stress conditions (i.e., activity test) was displayed by bream larvae fed 22:6(n-3)-enriched live feed.[130]

With turbot (*Scophthalmus maximus*) (n-3)HUFA enrichment has its most significant effect on larval pigmentation. However, survival remains too low and variability among experiments too high to evaluate the effect of dietary (n-3)HUFA levels on growth and survival.[131]

For the Pacific species, similar tendencies to those of the European species have been determined. Survival at metamorphosis and stress resistance (i.e., salinity shocks) in Asian sea bass (*Lates calcarifer*) is strongly correlated with the HUFA levels of its *Artemia* diet.[22] After 3 weeks culturing, milk fish (*Chanos chanos*) showed significant increases in growth (length and dry weight) when fed HUFA-fortified *Artemia*.[132] Similarly, rabbit fish larvae (*Siganus guttatus*) fed HUFA-rich *Artemia* displayed less mortality when disturbed than did controls fed HUFA-poor *Artemia*.[132]

Until early 1988, culture trials with mahi-mahi larvae (*Coryphaena hippurus*) had only been successful when they were fed natural copepods or other zooplankton. Culture tests with newly hatched *Artemia* did not prove successful.[133-135] In 1988 and 1989, significant progress in the larviculture of this fast-growing aquaculture candidate was achieved by various research groups in the U.S. and Australia. Feeding the larvae with (n-3)HUFA super-fortified *Artemia* resulted in more consistent larviculture outputs in terms of survival, larval growth, and health as compared to those cultured with the copepod *Calamoecia* or other zooplankton as food.[22,135]

Rearing of red drum larvae (*Sciaenops ocellatus*) is currently being studied in the U.S. Gulf Coast states. Rotifers are the first food, followed by *Artemia* nauplii,[136] and experiments on the effects of enrichment of both live foods are currently being conducted.[137]

D. FRESHWATER FISH CULTURE

Much freshwater fish culture is carried out in ponds with natural zooplankton as the larval food. The salmonids, perhaps the group cultured most widely on an intensive basis, have a relatively well-developed digestive tract at first feeding and are usually fed prepared diets immediately. Thus, *Artemia* is not used in the vast majority of freshwater fish culture. However, several species of freshwater fish are fed *Artemia*. Whitefish larvae (family Coregonidae) are often fed *Artemia* until they metamorphose and can be switched to a dry diet.[52] Hokanson and Lien[138] compared survival and growth of walleye larvae (*Stizostedion vitreum*) raised on diets of *Artemia*, natural zooplankton, and fish larvae and concluded that *Artemia* is the best choice for a first food. Barrows[139] suggested a 15-d feeding period on brine shrimp for walleye larvae prior to the feeding of traditional artificial diets. In the U.S. *Artemia* nauplii are increasingly used as a first food for striped bass larvae (*Morone saxatilis*).[140] Interestingly, although these fish are reared in fresh or very low-salinity water, recent evidence[141] suggests that they may have the fatty-acid requirements of a marine fish, which they become as adults. The larvae are typically fed *Artemia* from about 5 d post-hatch until about 20 d, then weaned onto an artificial diet until about 30 d, after which *Artemia* feeding ceases.[142]

A major drawback in feeding *Artemia* to freshwater organisms is that the *Artemia* die after 30 to 60 min in fresh water. Therefore, they are not continuously available to the predator, as they would be in marine systems, and must be fed intermittently every 2 to 3 h. A lot of the convenience of *Artemia* is thus lost, especially since dead *Artemia* may foul the water.

E. AQUARIUM FISH

As mentioned above, both live and frozen adult *Artemia* are used as food for species

reared as pets by home aquarists. Cysts are also purchased by these users and hatched for feeding as nauplii. Survival, vigor, and pigmentation were reported to be significantly improved in several tropical species when (n-3)HUFA levels were increased.[150] Although the quantities used by a pet fish owner are small compared to those used by a commercial aquaculturist, there are many more of the former than the latter.

F. TOXICOLOGICAL RESEARCH

An increasing market for *Artemia* cysts is developing among researchers in aquatic toxicology. As governmental requirements for environmental testing increase, many more tests are being conducted with many more species. Standards now exist for the use of *Artemia* nauplii as food for those organisms[143] and the availability of reference *Artemia* diets[144-146] greatly assists in the rigorous conduct of that testing.

VII. CONCLUDING REMARKS

Artemia usage in aquaculture is currently undergoing great changes. As new aquaculture species are developed and as aquaculture production increases, the demand for *Artemia* continues to grow, especially in Asia, Latin America, and Europe. At the Second International Symposium on *Artemia* in 1985, projections were made that cyst sales would increase to 150—170 tons annually before the year 2000.[42] Only three years later, at the World Aquaculture Society meeting, participants estimated that 1988 consumption would already be 250 tons. As demand increases, prices are likely to increase also.

The development of products and techniques to enrich *Artemia* has meant that cysts from the world's major production source, Great Salt Lake, could be used for marine as well as freshwater species. This has helped ease the shortage of cysts that inherently have an adequate fatty-acid profile for marine organisms.

Two major questions exist at this point. First, will *Artemia* supply be able to keep up with demand? Research is currently being conducted to try to understand the processes in extensive production of *Artemia*, so that someday salinas and lakes might be biologically managed to optimize production. Second, can artificial formulated diets ever totally replace *Artemia* as a sole food for cultured larvae? Research is being conducted both on the development of diets and materials to bind or encapsulate them and on the physiological and nutritional requirements of larvae. It appears at this point that adequate diets will not be ready for several years, if ever. For the time being, *Artemia* will continue to be the major live food for larviculture and, therefore, a product that unites aquaculturists and researchers in both developed and developing countries.

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